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ACCLIMATION OF FISH TO LOW OXYGEN

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ACCLIMATION OF FISH TO LOW OXYGEN

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by

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INTRODUCTION

This study was undertaken to assess the extent of metabolic adaptation exhibited by fish exposed for relatively short periods of time to low levels of oxygen. Extensive research is being carried out concerning the effects of low oxygen on growth, survival, etc., of fishes, but less is known of the capacity of fish to adapt metabolically to reduction in dissolved oxygen. Metabolic parameters investigated in the present study are metabolic rate of the whole animal, oxygen consumption of selected tissues, activity levels of certain respiratory enzymes, and levels of energy stores. Red blood cell counts were taken and measurements made of hemoglobin concentration and hematocrit (packed cell volume).

Most of the study was done on the bluegill sunfish (Lepomis macrochirus). A few experiments were performed with other centrarchid fishes; namely, white crappie (Pomoxis annularis), yellow perch (Perca flavescens), and pumpkinseed (Lepomis gibbosus). The choice of bluegill sunfish as the main experimental animal was due to two factors; (1) ease of maintenance in the laboratory; and (2) the fact that this species (and many other centrarchids) are exposed to periodic fluctuations in dissolved oxygen in their natural environment, and might be expected to possess mechanisms of physiological adaptation to oxygen stress.

The low oxygen acclimation level chosen was 1.2-1.4 cc/L.

(1.71-2.00 mg/L.). Preliminary experiments on the relationship of standard metabolic rate to oxygen concentration in bluegill sunfish indicated that this range marked the transition from the zone of respiratory independence (metabolic rate independent of oxygen concentration) to the zone of respiratory dependence (metabolic rate dependent on oxygen concentration). The transitiion point is sometimes called the Critical Oxygen Tension. There is as yet no convincing evidence for correlations between this parameter and ability of fish to tolerate low oxygen, and the choice of acclimation level is thus seen to be somewhat arbitrary. It is likely, however, that below the critical oxygen tension a fish is obtaining at least some of its energy anaerobically (unless of course it greatly reduces its total energy demands). It was our intention to test the hypothesis that prolonged exposure to a level of oxygen near the critical oxygen tension would result in adaptations leading to increased metabolic efficiency in the face of oxygen stress.

The following persons have assisted in carrying out this study. All participated as research assistants during the period indicated.

Robert L. Puyear	7/64 - 9/64
Thomas I. Ihrig	7/64 - 9/65; 7/66 - 9/66
Steven E. Meredith	7/65 - 9/65
Hans W. Behrisch	7/65 - 9/66

METHODS AND PROCEDURES

Most of the fish used in the study were caught by hook and line from Triangle Lake, about 40 mi. from Corvallis, and transported to the laboratory in styrofoam chests. Barbless hooks were used and only a small number of specimens were injured sufficiently to necessitate discarding them. In the laboratory the fish were kept in fiberglass lined wooden tanks supplied with running dechlorinated tap water, and fed a standard fish pellet. Generally it takes about a week for these fish to begin feeding on this ration. Animals were left in the tanks for a minimum of two weeks, or until actively feeding, before being used in experiments.

Fish used in the winter 1965 experiments were seined from Oregon State Game Commission rearing ponds located near St. Louis, Oregon. The average size of these specimens was much larger than those from Triangle Lake.

Temperatures in holding tanks ranged from 19-22 degrees C. in summer, about 14-17 degrees in fall, and 9-11 degrees in winter. Experiments in the summer were performed at 20 degrees, in the fall at 15, and in the winter at 10 degrees C.

Experimental Protocol:

Fish are removed from the stock tanks, placed in 5 gal. aquaria (2-3 fish per aquarium) and left overnight with air-saturated water flowing through the tanks. Each aquarium is covered with a loose

fitting glass plate. At the start of the experiment, the water supply to half the tanks is switched to a source of low oxygen water maintained at a concentration of 1.2-1.4 cc/L. It takes 3-4 hours for the oxygen concentration in the aquaria to fall to the acclimation level. The fish in the remaining tanks serve as controls.

The method of reducing the oxygen content involves passing water through a long plexiglass column into which nitrogen gas is bubbled at a controlled rate to reduce the oxygen to the desired level. The plexiglass column is filled with Raschig rings to break up the flow of nitrogen gas. Water leaving the column is led through an overflow jar from which lines lead to the individual acclimation tanks. With this arrangement we are able to maintain the oxygen concentration in the acclimation tanks within 0.2 cc/L. for an indefinite period.

At the end of two or six days of acclimation all fish were sacrificed and blood and tissue analyses carried out according to the plans for a particular experiment.

Blood Analysis:

Blood is sampled by carefully inserting a heparinized capillary tube into the common cardinal vein at the caudal margin of the gill arches and allowing several drops to flow onto a small piece of Parafilm. Aliquots are then pipetted for the various analyses.

Red blood cell counts were done on a standard Neubauer double-ruled counting slide, using Yokoyamas diluent. Hematocrit was

estimated using heparinized capillary tubes with a microhematocrit centrifuge. Hemoglobin was determined on 0.02 mL. samples using the pyridine haemochromogen method as described by Anthony (1960).

Blood glucose was determined using GLUCOSTAT prepared reagent (Worthington Biochemical Corp.). The procedure is specific for glucose. Lactate was determined enzymatically using the kit supplied by Sigma Chemical Co. Readings were made at 366 mu. using a Beckman DU Spectrophotometer.

Glycogen Analysis:

Samples of dorsal epaxial muscle and of liver were removed quickly and immediately placed in 30% KOH for digestion. Muscle and liver glycogen were determined as glucose after alcohol precipitation and acid hydrolysis. Estimation of glucose in the acid hydrolyzate was done by the phenol method, following the procedure of Montgomery (1957).

Oxygen Consumption of Tissues:

Gill, liver and muscle tissue samples were quickly removed, sliced freehand in the cold (with the exception of gill, in which the arches were simply separated and used whole) and suspended in a balanced salt solution for fish tissues described by Holmes (1960). Oxygen consumption was measured in Warburg flasks following the standard procedures for manometric analysis. Results are reported as microliters oxygen consumed/gram dry weight/hour.

Enzume Activity:

Muscle and liver samples were removed and immediately homogenized in ice cold distilled water. The homogenates were made up to 10 mL., centrifuged, and enzyme activity determinations made on the supernatant. Total LDH activity in muscle and liver was estimated by the Berger-Broida colorimetric method (Sigma Chemical Co., Tech. bull. No. 500) with readings made at 475 mu. Muscle aldolase was estimated colorimetrically by the Sigma modification of the Sibley-Lehninger method (Sigma Chemical Co., Tech. Bull. No. 750), with readings made at 540 mu. All aldolase and LDH activity measurements are reported in terms of activity/mg. protein. Protein was determined on one mL. aliquots of the water supernatant, using the quantitative Biuret procedure.

Muscle and liver cytochrome oxidase activity was determined manometrically, using the procedure as outline in Umbreit et al. (1964). Results are reported as microliters oxygen consumed/gram dry weight/hour.

Protocol for Metabolic Rate-Oxygen Concentration Experiments:

Oxygen consumption rates of whole animals were determined in a constant-flow apparatus. Rates were calculated from the difference in oxygen content of water entering and leaving the respirometer flasks, the rate of water flow, and wet weight of animals. Since several hours were required for fish to adjust to the confinement of

the respiration flasks, they were transferred from the acclimation tanks to the respirometer apparatus the night before their allotted acclimation time was reached, and maintained in the apparatus at the acclimation oxygen level. At the start of a metabolic rate run the apparatus was flushed with air-saturated water. When a stable oxygen level was achieved, one hour was allowed to elapse and then two consecutive measurements, one half hour apart, were taken of oxygen consumption. The oxygen level in the apparatus was then lowered and the procedure repeated.

RESULTS AND DISCUSSION

Metabolic Rate vs. Oxygen Concentration:

The most extensive series of experiments were performed on bluegill sunfish. The results of metabolic rate determinations on intact animals are shown in figure one. Oxygen values on the abcissa are given in mmHg so the scales may coincide in comparing the metabolic rate-oxygen concentration relation for the various temperatures. Each value on the graphs represents the mean of measurements on 2-10 fish. Lines through the points are drawn by eye and represents trends only.

The fish used ranged from 20-50 grams in size, with the exception of four fish used in the winter 1965 experiments which ranged from 90-114 grams. A more accurate representation of metabolic rate might result from correcting for the effect of size on respiration,

but it was felt that the number of fish used was too small and the size range too limited to make feasible a plot of body size versus respiration for each of the oxygen concentrations tested.

Our principal interest was in the effect of low oxygen acclimation on the metabolic rate. Figure one indicates that at 20 degrees exposure to low oxygen depresses standard metabolic rate, compared with controls in air-saturated water, over at least part of the range of oxygen partial pressures. There is also a slight shift of the critical oxygen tension to a lower value. Both might be considered adaptive responses in fish subjected to oxygen stress in their natural environment. Somewhat less lowering of metabolic rate was observed at 15 degrees, and almost no difference between controls and low oxygen acclimated fish was found at 10 degrees. At none of the three temperatures does acclimation to low oxygen appear to shift the metabolic rate appreciably in the zone of respiratory dependence. Beamish (1963) reports similar findings in carp and goldfish at 20 degrees, excepting that acclimation to low oxygen caused a more pronounced fall in metabolic rate in his fish than we observed in bluegill.

In the fish tested at 20 degrees, there is an indication (especially in those acclimated to air-saturated water) that standard metabolic rate increases at intermediate oxygen levels. This has been noted previously by VanDam (1939) in rainbow trout and more recently by Beamish (op. cit.). These authors suggest that the increased

metabolic rate may be attributed to a greater cost of respiration occasioned by an increase in the respiratory volume (amount of water flowing through the gills per minute). It is interesting that bluegill previously exposed to low oxygen show this "compensatory increase" in metabolic rate to only a very limited extent (figure one). At 15 and 10 degrees there does not appear to be a rise in metabolic rate near the critical oxygen tension (figure one). Likewise, Beamish (op. cit.) reports the absence of a "compensatory increase" in metabolic rate at 10 degrees in both carp and goldfish.

Fewer experiments were performed on the remaining species and in some cases only one temperature, or only fish from air-saturated water, were investigated. Some interesting comparisons, nevertheless, can be made with the results obtained on bluegill. Figure two shows the results of experiments on pumpkinseed, a species closely related to bluegill, at 20 degrees. The critical oxygen tension range agrees closely with that for bluegill, and there is an indication that standard metabolic rate in the low oxygen acclimated fish is depressed at the higher oxygen tensions. Metabolic rates at all oxygen tensions, however, are considerably higher than in the bluegill sunfish. This could mean that pumpkinseed are more excitable than bluegill and require a much longer time in the respirometer before standard rates are obtained or, alternatively, it could suggest a real difference in standard metabolic rates between

the two species. Since a total of only four fish were tested, the results must be considered as preliminary.

A series of metabolic rate experiments at 20 degrees on yellow perch is reported in figure three. The fish acclimated to air-saturated water show an unusual pattern of response. As the oxygen tension is lowered metabolic rate increases markedly, reaching at 50% saturation a rate almost double that in air-saturated water. As the oxygen tension is lowered further, metabolic rate begins a steady decline. There is apparently, therefore, no range of oxygen tensions over which the metabolic rate is relatively constant--i.e. zone of respiratory independence. By contrast, the fish previously acclimated to low oxygen show only a slight rise in metabolic rate as the oxygen tension islowered below saturation. Over most of the range in oxygen partial pressures tested, the rate of oxygen consumption is considerably below that of the controls acclimated to air-saturation. The critical oxygen tension seems to be between 30-40 mmHg. agreeing with that for the low oxygen acclimated bluegill (figure one). Below the critical tension, in the zone of respiratory dependence, metabolic rate of the low oxygen acclimated group is almost identical to the group from air-saturated water, again agreeing with the results on bluegill.

Metabolic rate-oxygen tension curves were obtained for white

crappie at 20 and 15 degrees. Unfortunately this species did not survive acclimation to 1.4 cc/L. oxygen and a curve for low oxygen acclimated animals has not yet been obtained. The curve for the fish from air-saturated water at 20 degrees indicates a zone of respiratory independence where metabolic rate remains about the same level, but the critical oxygen tension, somewhere between 60-80 mmHg, is much higher than in bluegill. The curves obtained at 15 degrees are very similar to those obtained on bluegill at the same temperature.

A striking feature of all the experiments, irrespective of species, acclimation state, or temperature, is the tendency for metabolic rates to converge to a common value below the critical oxygen tension, in the zone of respiratory dependence, suggesting that metabolic rate is diffusion dependent at these very low oxygen partial pressures.

Several species differences in patterns of response to intermediate oxygen levels have been shown and will form the basis of future comparative studies on centrarchid fishes.

Tissue Oxygen Consumption:

Values of tissue oxygen consumption for selected tissues from control and low oxygen acclimated bluegill at 20 degrees are given in Table one. After two days acclimation there is an increase in oxygen consumption in all tissues, but only in gill tissue is the difference statistically significant. After six days acclimation some decrease

in gill tissue respiration (not statistically significant) was noted, but the small number of control fish sampled does not lend much confidence to the mean value reported. Unfortunately no values of liver tissue respiration are available for the six day acclimation period.

Since the direction of change in oxygen consumption of all tissues examined is opposite to that of the whole animal after acclimation, it would seem that changes in tissue respiration is not one of the things responsible for the observed depression in whole animal metabolic rate.

Tissue oxygen consumption values for bluegill at 10 degrees are given in Table two. After two days acclimation to low oxygen, all tissues show increases in oxygen consumption, attaining borderline significance in the case of gill and liver. After six days of acclimation the mean values for control and experimental fish are practically identical.

Gill and liver tissue respiration are considerably lower than values from summer fish, as expected from the temperature change. The respiration of muscle tissue, however, is actually slightly higher in winter, indicating some metabolic overcompensation for temperature. This response of muscle tissue has been observed in other studies in our laboratory on metabolic rate of bluegill in relation to body size (H. Behrisch and A. W. Pritchard, Can. J. Zool., In Press).

In both the 10 and in the 20 degree experiments, the variability

of muscle tissue respiration from experimental animals greatly exceeds that in control animals. We cannot, as yet, offer an explanation.

Respiration rates of tissues from white crappie were obtained only at 20 degrees, on animals acclimated for two days to low oxygen. The results are given in Table three. Exposure to low oxygen produced no significant change in gill or liver tissue respiration. Muscle tissue from experimental fish, however, respired at a lower rate than controls (borderline significance), which contrasts with the results obtained on bluegill. We do not know yet if acclimation to low oxygen also reduces the oxygen consumption of the whole animal.

Enzyme Activities:

Enzyme activity measurements (Table four) show a great deal of variability. The measurements were carried out on crude tissue homogenates rather than purified enzymes. Further, pH optima were not run on the extracts and although a shifting of the pH optimum for the enzyme associated with acclimation is not likely, this is a valid criticism. With these limitations, we may note some interesting trends in the data of Table four.

Both liver and muscle cytochrome oxidase activities are decreased as a result of two days acclimation to low oxygen. In the case of muscle the decrease is statistically highly significant. Liver and muscle LDH activities increase slightly after acclimation but in neither two or six day exposure periods are the differences statistically significant.

Muscle aldolase activities increase (borderline significance) after both two and six day exposures to low oxygen. Since the latter two enzymes are involved in glycolysis and cytochrome oxidase is concerned with transport of electrons to oxygen, the results are consistent with the hypothesis that acclimation to low oxygen increases the extent of anaerobiosis in these fish, concomittantly decreasing the extent of cellular oxidation. The results are not consistent with those on tissue respiration (Table one) where it is noted that both liver and muscle show an increase in respiration rate upon acclimation to low oxygen.

The six day results for cytochrome oxidase present a confusing picture. In liver there is a slight decrease in activity (not significant) but in muscle there is a marked (but not statistically significant) increase. In the latter case the number of control animals used is very small and the variability of the data from the low oxygen acclimated animals unusually large.

Energy Stores:

Glycogen is a metabolically labile substance, being readily broken down by the liver into glucose and anaerobically in muscle to lactate. From Table five we note that after two or six days of acclimation to low oxygen the amount of glycogen in both muscle and liver does not differ significantly from controls, indicating neither net utilization or synthesis of glycogen as a result of acclimation

to low oxygen. This represents only an end result of course, and tells us nothing about rapidity of turnover of glycogen in the intervening period. Heath and Pritchard (1965) report that muscle glycogen is depleted by 60% and liver glycogen by 75% immediately following exposure of bluegill to severe hypoxia, indicating rapid mobilization. Resynthesis of glycogen to near normal levels occurs within 24 hours following severe hypoxia (op. cit.).

Both control and experimental glycogen levels are somewhat reduced after six days exposure, as compared with two days (Table five). This may represent a "starvation" effect since neither group of animals were fed during the experiment.

In Table six are presented the results of blood glucose and blood lactate analyses following two days acclimation to low oxygen. Blood glucose levels are essentially the same in experimental and control groups. There is, however, a significant increase in blood lactate in experimental animals perhaps reflecting increased reliance on anaerobiosis.

Red Blood Cell Counts, Hematocrit, and Hemoglobin:

Data for the various hematological parameters is summarized in Tables seven and eight. At 20 degrees all three things measured increase after two or six days acclimation to low oxygen (Table seven). Significant increases were found for RBC counts after two and six days acclimation, and for hemoglobin after six days exposure.

Although the increase in RBC count (over controls) in fish exposed to low oxygen for six days is greater than for fish exposed for two days, only borderline significance could be demonstrated. This probably reflects the relatively small sample sizes, and great variability in values from experimental animals.

In winter animals, tested at 10 degrees, there is an increase in all parameters but the increase is statistically significant only in the case of RBC counts from fish exposed to low oxygen for two days.

These data suggest increased hematopoiesis in fish exposed to low oxygen, which would presumably enhance the oxygen capacity of the blood. In summer fish, tested at 20 degrees, two days exposure is apparently not sufficient to trigger increased synthesis of hemoglobin (Table seven). After six days, however, the experimental animals show a highly significant increase over controls.

It is interesting that in winter animals (Table eight) both hematocrit, representing packed cell volume, and hemoglobin are lower than in summer fish; whereas RBC counts are, if anything, slightly higher. This would suggest increase numbers of <u>smaller</u> red blood cells, containing less hemoglobin, in the winter animals. A thorough study of seasonal variation in hematopoiesis in these fish should prove rewarding.

CONCLUSIONS

From this study we conclude that exposure to low oxygen slightly lowers the standard metabolic rate of bluegill sunfish in the range of oxygen concentrations above the critical oxygen tension. The effect of acclimation is greatest at 20 degrees and progressively less at 15 and 10 degrees. Below the critical oxygen tension, at all temperatures, metabolic rates decline sharply and tend to converge towards a common value. Prior exposure to low oxygen has little or no effect on metabolic rate in this low range of tensions.

An enhanced anaerobic metabolism in animals exposed to low oxygen is suggested by the increased activity levels of total LDH and aldolase together with decreased activity of ytochrome oxidase. Blood lactate is also increased significantly in the low oxygen acclimated fish.

The increases in RBC counts, hematocrit and especially hemoglobin suggest enhanced oxygen capacity in the low oxygen acclimated animals.

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Figure one. Relationship of metabolic rate to oxygen tension in bluegill sunfish. Circles, 20 degrees; Triangles, 15 degrees; Squares, 10 degrees. Open symbols, acclimated to air-saturated water; Closed symbols, acclimated to low oxygen.

Figure two. Relationship of metabolic rate to oxygen tension in pumpkinseed. Temp: 20 degrees. Open circles, acclimated to air-saturated water; Closed circles, acclimated to low oxygen.

Figure three. Relationship of metabolic rate to oxygen tension in yellow perch. Open circles, acclimated to air-saturated water; Closed circles, acclimated to low oxygen.

Figure four. Relationship of metabolic rate to oxygen tension in white crappie. Open circles, acclimated to air-saturated water, 20 degrees; Open triangles, acclimated to air-saturated water, 15 degrees; Closed triangles, acclimated to low oxygen, 15 degrees.

Fig. 1. Bluegill Sunfish

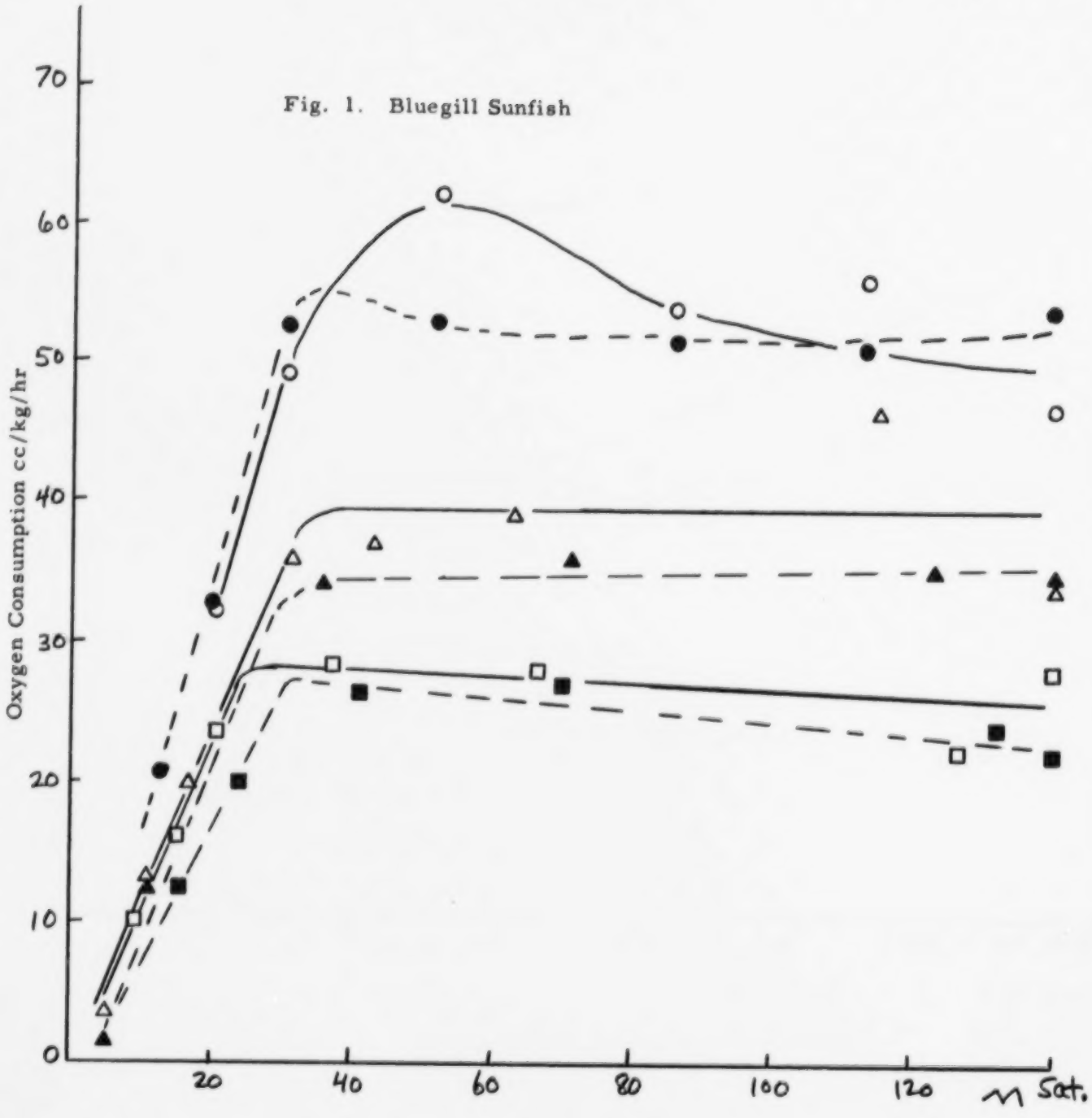

Fig. 2. Pumpkinseed

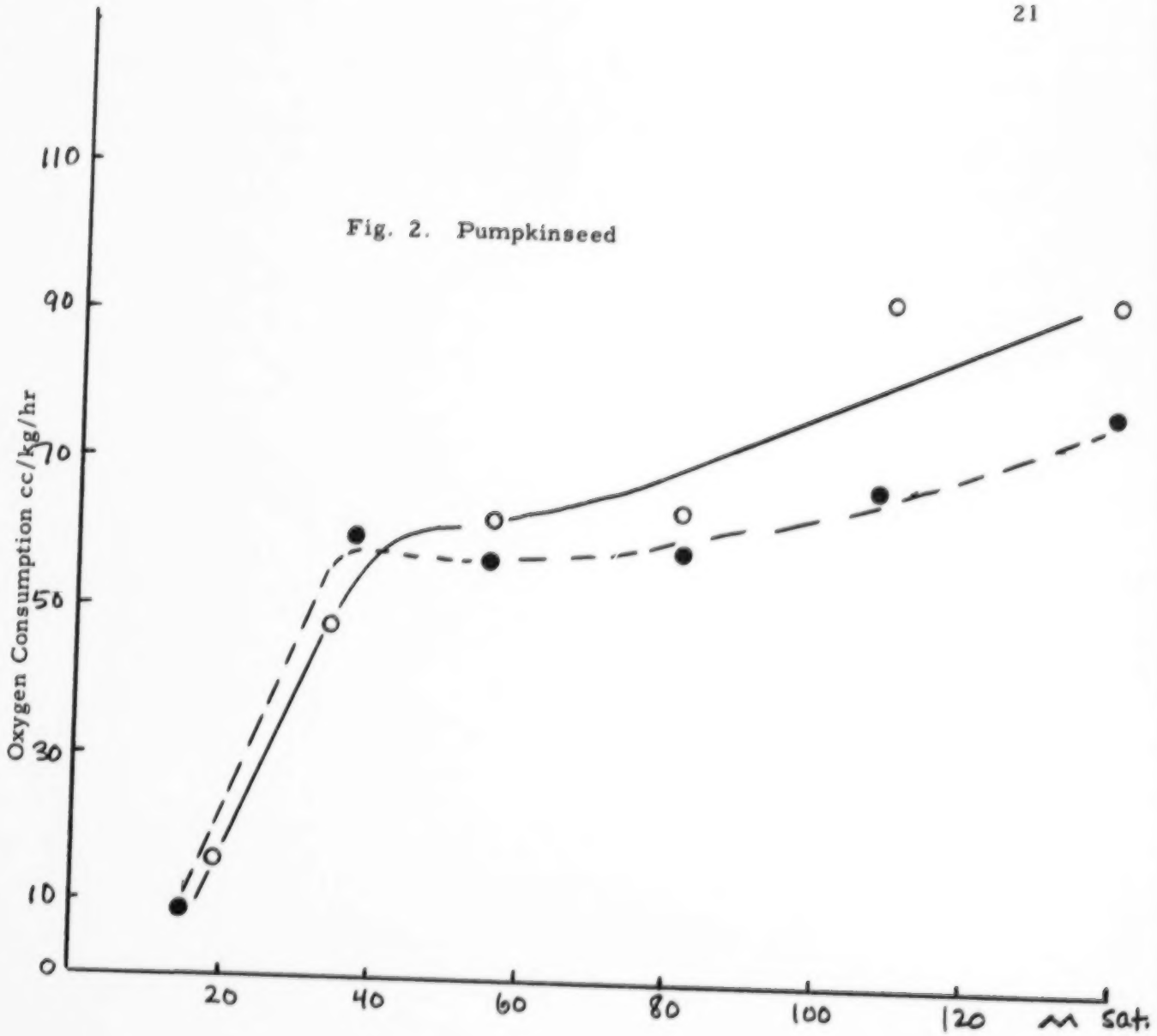

Fig. 3. Yellow Perch

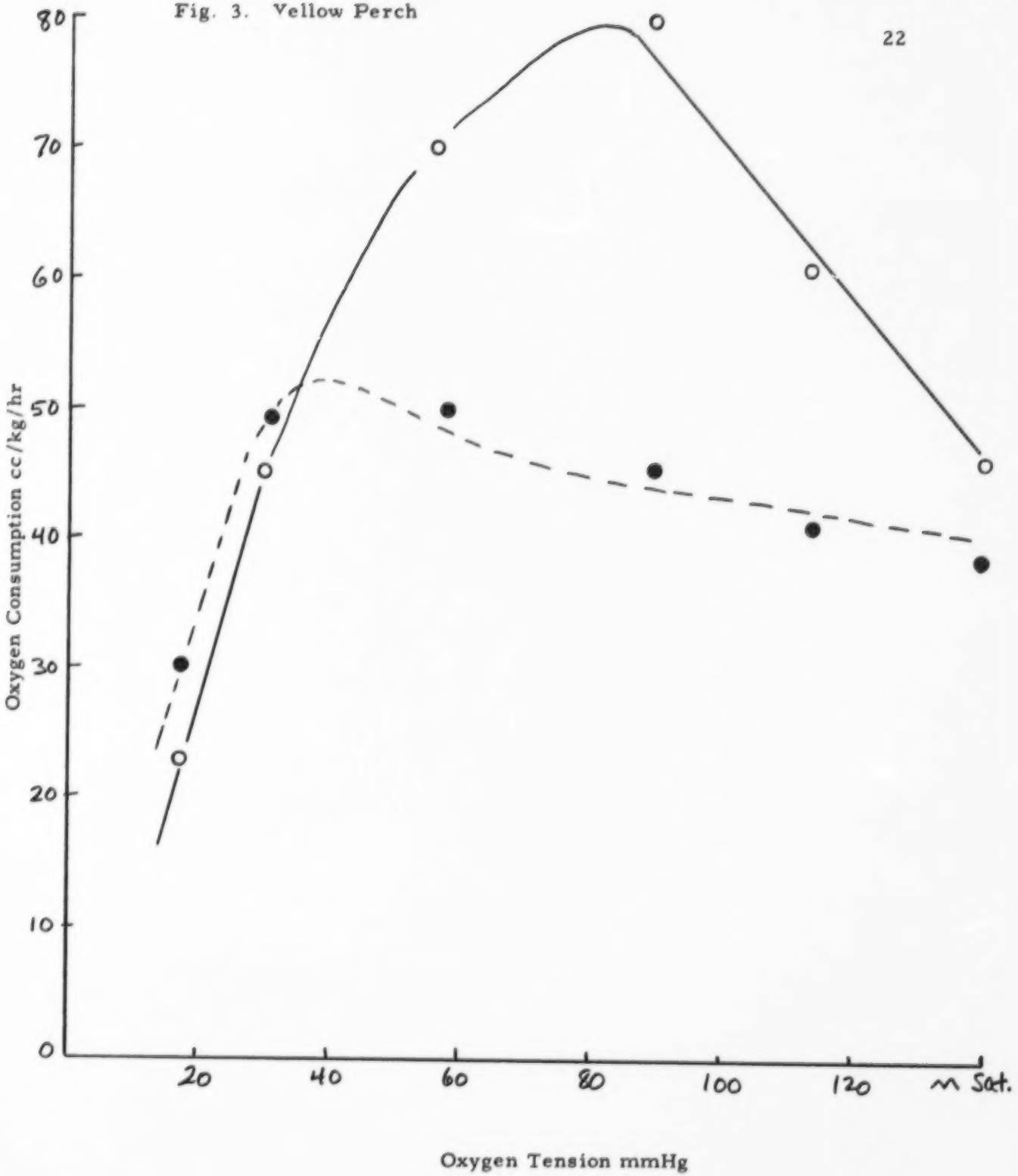

Fig. 4. White Crappie

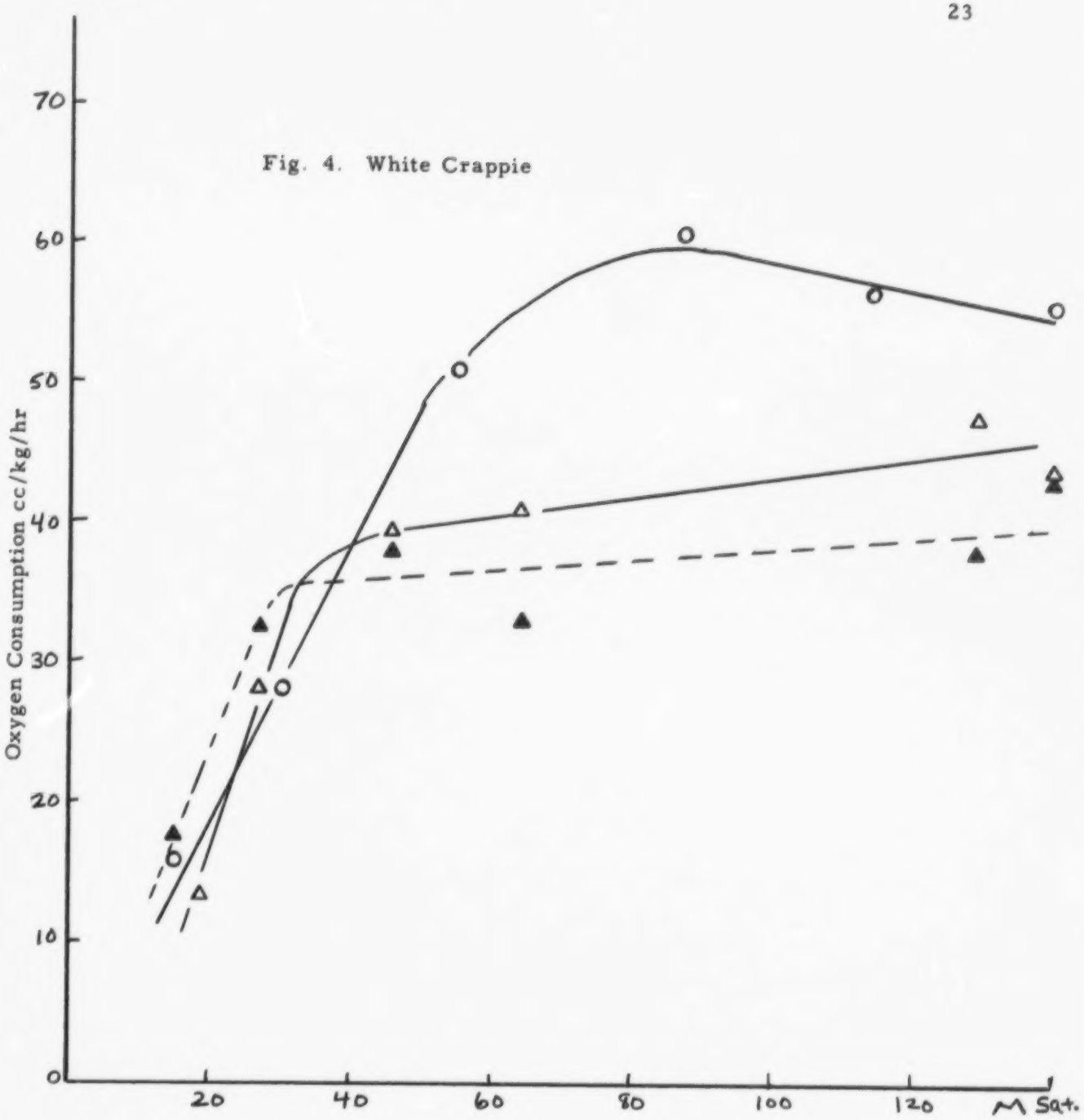

Table 1. Effect of acclimation to low oxygen on O_2 consumption of selected tissues in bluegill sunfish. Units: microliters/gm. dry wt. /hour. Values reported as mean ± S. E Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc O_2/L. Controls maintained in air-saturated water. Temp. 20°C

	Length of Acclimation	Control	Experimental	"t" Value	Significance
Gill	2 days	976 ±124 (18)	1318 ±127 (24)	1.88	P < .1 Borderline
	6 days	1003 ±206 (3)	798 ±72 (8)	1.24	P > .2 N. S.
Liver	2 days	808 ±94 (18)	1035 ±122 (21)	1.43	P > .2 N. S.
Muscle	2 days	358 ±32 (18)	406 ±62 (24)	.60	P > .5 N. S.
	6 days	270 ±34 (6)	388 ±63 (6)	.52	P = .6 N. S.

Table 2. Effect of acclimation to low oxygen on oxygen consumption of selected tissues in bluegill sunfish. Units: microliters/gm dry wt./hour. Values reported as mean ± S.E. Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc/L. Controls maintained in air-saturated water. Temp.: 10°C.

	Length of Acclimation	Control	Experimental	"t" Value	Significance
Gill	2 days	378 ±26 (8)	461 ±33 (9)	1.96	P < .1 Borderline
	6 days	421 ±27 (6)	422 ±16 (7)	.033	P > .9 N. S.
Liver	2 days	351 ±75 (8)	469 ±43 (12)	1.80	P < .1 Borderline
	6 days	404 ±65 (7)	411 ±38 (11)	.10	P > .9 N. S.
Muscle	2 days	377 ±38 (8)	415 ±47 (12)	.58	P > .5 N. S.
	6 days	410 ±43 (7)	402 ±42 (11)	.13	P = .9 N. S.

Table 3. Effect of acclimation to low oxygen on oxygen consumption of selected tissues in white crappie. Units: microliters/gm dry wt./hour. Values reported as mean ± S.E. Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc/L. Controls maintained in air-saturated water. Temp.: 20°C.

	Length of Acclimation	Control	Experimental	"t" Value	Significance
Gill	2 days	742 ±47 (6)	744 ±52 (8)	.027	P > .9 N.S.
Liver	2 days	839 ±112 (6)	874 ±61 (8)	.295	P > .7 N.S.
Muscle	2 days	230 ±28 (6)	173 ±14 (8)	1.94	P < .1 Borderline

Table 4. Effect of acclimation to low oxygen on activity of selected enzymes. Units: Cytochrome oxidase, μl/gm dry wt/hour; LDH, Berger-Broida units/mg protein; Aldolase, S-L units/mg protein. Values reported as mean, ∓ S.E. Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc O_2/L. Controls maintained in air-saturated water. Temperature: 20°.

	Length of Acclimation	Control	Experimental	"t" Value	Significance
Liver Cytochrome Oxidase	2 days	1372 ±130 (4)	1062 ±128 (8)	1.52	P > .1 N. S.
	6 days	1353 ± 237 (5)	1217 ±128 (10)	.55	P = .6 N. S.
Muscle Cytochrome Oxidase	2 days	1440 ± 35 (5)	920 ±129 (7)	3.32	P < .01 highly significant
	6 days	1140 ±58 (3)	1551 ±311 (5)	.987	P > .3 N. S.
Liver LDH	2 days	168.8 + 45 (10)	173.8 ± 38 (16)	.083	P > .9 N. S.
	6 days	142.2 ± 28 (4)	229.4 ±53 (10)	1.01	P > .3 N. S.
Muscle LDH	2 days	1412 ±171 (10)	1632 ±138 (16)	.762	P > .4 N. S.
	6 days	1314 ± 53 (5)	1452 ±105 (10)	.887	P = .4 N. S.
Muscle Aldolase	2 days	75.9 ± 21 (10)	127.8 ±19 (15)	1.81	P < .1 Borderline
	6 days	62.0 ±7.6 (5)	130.2 ±25 (10)	1.87	P < .1 Borderline

Table 5. Effect of acclimation to low oxygen on glycogen levels in muscle and liver of bluegill sunfish. Units: mg per 100 gm wet weight. Values reported as mean ± S. E. Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc O_2/L. Controls maintained in air-saturated water. Temperature: 20°.

	Length of Acclimation	Control	Experimental	"t" Value	Significance
Liver Glycogen	2 days	4434 ±340 (8)	4457 ±450 (14)	.0003	P> .9 N. S.
	6 days	3881 ±1210 (4)	3978 ±598 (5)	.076	P> .7 N. S.
Muscle Glycogen	2 days	85.6 ±17.9 (9)	91.3 ±15.8 (16)	.322	P> .9 N. S.
	6 days	43.2 ±6.8 (5)	57.9 ±10 (5)	1.21	P> .2 N. S.

Table 6. Effect of acclimation to low oxygen on blood glucose and blood lactate levels in bluegill sunfish. Units: mg per 100 cc plasma. Values reported as mean ±S. E. Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc O_2/L. Controls maintained in air-saturated water. Temperature: 20°.

	Length of Acclimation	Control	Experimental	"t" Value	Significance
Blood glucose	2 days	52.8 ±5.66 (5)	52.7 ±3.36 (9)	.016	P > .9 N. S.
Blood Lactate	2 days	5.50 ±.32 (7)	7.37 ±.69 (8)	2.33	P < .05 Significant

Table 7. Effect of acclimation to low oxygen on red blood cell count, hematocrit and hemoglobin concentration in bluegill sunfish. Units: RBC count, no. of cells per cu. mm.; hematocrit, per cent packed cells; Hemoglobin, gm per 100 ml. whole blood. Values reported as mean ± S E. Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc/L. Controls maintained in air-saturated water. Temp: 20° C.

	Length of Acclimation	Control	Experimental	"t" Value	Significance
RBC Count	2 days	213 ±13 (13)	251 ±6.8 (29)	2.80	P < .01 Highly significant
	6 days	214 ±9.9 (9)	272 ±29 (9)	1.88	P < .1 Borderline
Hematocrit	2 days	32.1 ±1.8 (13)	35.5 ±1.1 (28)	1.67	P = .1 N.S.
	6 days	30.9 ±1.0 (9)	34.5 ±2.3 (7)	1.56	P > .1 N.S.
Hemoglobin	2 days	6.53 ±.4 (13)	6.95 ±.28 (29)	.84	P = .4 N.S.
	6 days	6.50 ±.27 (9)	7.80 ±.35 (9)	2.94	P < .01 Highly Significant

Table 8. Effect of acclimation to low oxygen on red blood cell count, hematocrit and hemoglobin concentration in bluegill sunfish. Units: RBC count, no. of cells per cu. mm.; hematocrit, per cent packed cells. Hemoglobin, gm. per 100 ml. whole blood. Values reported as mean ± S.E. Numbers in parentheses indicate number of fish sampled. Acclimation level: 1.2-1.4 cc/L. Controls maintained in air-saturated water. Temp: 10°C.

	Length of Acclimation	Control	Experimental	"t" Value	Significance
RBC Count	2 days	248 ±9.1 (10)	299 ±13 (21)	2.53	P < .01 Highly significant
	6 days	224 ±32 (4)	279 ±18 (9)	1.58	P > .1 N.S.
Hematocrit	2 days	27.4 ±1.9 (10)	30.0 ±1.5 (20)	1.04	P > .3 N.S.
	6 days	25.6 ±2.9 (4)	30.1 ±1.9 (9)	1.32	P = .2 N.S.
Hemoglobin	2 days	5.43 ±.41 (8)	6.07 ±.28 (16)	1.32	P = .2 N.S.
	6 days	5.56 ±.62 (4)	6.16 ±.24 (9)	1.12	P > .2 N.S.